CHAPTER 4

Prevalence of split nerve fiber layer bundles in healthy eyes, imaged with scanning laser polarimetry

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Abstract

Purpose The GDx is a scanning laser polarimeter, designed to assess the peripapillary nerve fiber layer in vivo. On the GDx, a nerve fiber bundle can appear as a single, or as a split bundle. The aim of our study was to determine the prevalence of split nerve fiber layer bundles, and to demonstrate its clinical relevance.

Design cross-sectional study.

Participants 254 healthy volunteers participated.

Methods We imaged 454 eyes of 254 healthy caucasians with the GDx (Laser Diagnostic Technologies, San Diego, CA). All eyes had an intraocular pressure <=21 mmHg, normal appearance of the optic nerve head and normal visual fields (Humphrey Field Analyzer 24-2 full threshold program). According to our working definition, a bundle appeared 'split' when the color-coded pixels corresponding to areas of higher retardation were clearly divided into two more or less symmetrical parts, not resembling a wedge defect. The classification was performed by two independent observers who used an identical set of reference examples to standardize the classification.

Main outcome measures The presence of a split nerve fiber layer bundle.

Results Interobserver agreement was very good (kappa= 0.83) and a consensus was reached in all cases. In 419 eyes (92.3%), there was no split bundle. A split superior bundle was seen in 29 eyes (6.4%). A split inferior bundle was observed in 5 eyes (1.1%) and in 1 eye (0.2%) a split bundle was seen superiorly and inferiorly. When considering subjects, a split superior bundle (either in the right eye, or in the left eye, or in both eyes) occurred in 12.0% of normal subjects. The 'superior maximum' parameter was significantly lower in eyes with a split superior bundle than in eyes with a single superior bundle (67.2μ vs 89.9μ; $p<0.001$). The same was observed for the 'symmetry' parameter (0.88 vs 0.98; $p<0.001$).

Conclusions Split nerve fiber layer bundles are a common finding in healthy eyes when imaged with the GDx. A split superior bundle is the most occurring variation. In these last cases, an abnormal 'superior maximum' or 'symmetry' parameter, otherwise potential indicators of glaucoma, should not readily be interpreted as abnormal.

Introduction

The GDx (Laser Diagnostic Technologies, San Diego, CA) is a scanning laser polarimeter, that assesses nerve fiber layer (NFL) thickness in the peripapillary retina, and discriminates well between normals and glaucoma patients.[1-3] A detailed description of the GDx has been published elsewhere.[4-6] In short, a beam of polarized laser light is sent through the birefringent retinal NFL. As the light is reflected back into the instrument, the amount of phase shift (retardation) is determined and is thought to linearly reflect NFL thickness, as has been shown in a monkey model.[7] Anterior segment retardation is compensated by the instrument. In about 0.7 seconds the peripapillary retina is scanned at 65536 locations, and a retardation image of 256x256 pixels is constructed. This image is color-coded: a continuous scale from yellow to red to blue represents areas from high to low retardation. As adjacent pixels go from one color to another, the true difference in polarimetric reading may in fact be not as large as it seems.

The grouping of similarly colored, high-retardation pixels, when contrasted with surrounding pixels, gives the appearance of a 'bundle' and we will further refer to this grouping as such. Two such bundles are usually seen, typically superiorly and inferiorly. GDx data is characterized by a large interindividual variability.[3,4] Also the bundles that appear on the GDx vary in shape and thickness. We noticed earlier that some subjects with healthy eyes had an NFL orientation that we first believed indicated NFL defects. Observing this orientation more and more in healthy eyes, we came to believe it is a normal physiological finding. We have started to call this orientation 'split nerve fiber layer bundles', in contrast to single nerve fiber layer bundles that appear in the majority of healthy eyes. Split bundles can easily be distinguished from wedge shaped defects of the NFL, which have a markedly different appearance, as we will show.

The purpose of this study was to propose a definition for these split nerve fiber layer bundles, to investigate their prevalence in a population of healthy eyes, and to demonstrate their effect on some of the GDx parameters.

Methods

Measurement procedures A measurement session for one eye always consisted of obtaining a minimum of 3 single images of high quality (i.e. well focussed, the optic disc well centered in the image, equal and total illumination in all segments and no motion artifacts). These three images were then aligned and converted by the software into one 'mean image'. During all measurements, we saw to it that patients had their heads as upright as possible. Ambient lights were left on.

Areas of blood vessels are a source of noise[8] and are therefore automatically excluded for analysis by the GDx software. The entire image is divided into 4 segments centered on the optic disc: superior 120°, inferior 120°, nasal 50° and temporal 70°. For a quantitative approach, several parameters are available. In this study, the 'superior maximum' and the 'symmetry' parameter were considered. The superior maximum (smax) parameter is the average of the 1500 thickest pixels in the superior segment outside the green peripapillairy band. An inferior maximum parameter is defined likewise for the inferior segment. The symmetry parameter is the ratio superior maximum/inferior maximum.

Subjects After informed consent was obtained from the volunteers, 454 eyes of 254 healthy caucasians (age range 20-79 years; mean 51.6 years) were imaged with the GDx. Originally, 262 subjects were included for this study, but 8 (3.1%) of them were unsuitable for GDx imaging due to either inability to fixate or extremely large zones of peripapillary atrophy. All subjects were equally distributed across 10-year cohorts ranging from 20 to 80 years. All subjects were imaged on the same instrument within a time period of 6 months. They had no diabetes or hypertension that required medical treatment, nor any ocular history. All eyes had an intra ocular pressure <=21 mm Hg, normal appearance of the optic nerve head and normal visual fields (Humphrey Field Analyzer 24-2 full threshold program).

The split bundle Two independent observers, who used the same reference examples, classified the GDx printouts. Some of these reference examples have been presented in fig 4-1. The final classification was established by a consensus, which could be agreed upon in all cases. On the GDx, a nerve fiber bundle can appear as a single bundle (example 1), or as what we call a 'split bundle' (example 2). According to our working definition, a bundle appeared 'split' when the color-coded pixels corresponding to areas of higher retardation were clearly divided into two more or less symmetrical parts, not resembling a wedge defect. We will explain what we mean with 'clearly', 'symmetrical', and a 'wedge defect'.

With 'clearly', we mean that the split has to be 'deep enough'; the blue area separating the two 'legs' of the split bundle has to touch on the green peripapillary band, or extend even further towards the center of the image. In our experience, a single and a split nerve fiber layer bundle are two extremes on a continuous scale. This is partly illustrated in examples 2 and 3, that both meet the criteria of our definition of a split bundle. However, in example 2 the split is 'deeper'; in other words, the blue area separating both legs of the bundle extends further towards the center of the image than in example 3 (also note its effect on the double hump pattern). Descending further down the scale from split to single bundle is example 4, where the split is not 'deep enough' to meet the criteria of our definition. We called this a partially split bundle. This was also how the reference examples worked: any bundle that was split like this, or less, was not a split bundle by our definition.

The next part of our definition relates to symmetry. In examples 2 and 3, the two legs of the split bundle are equally large. Example 5 shows a split that is not symmetrical and has therefore not been classified as a split bundle according to our definition. Clinically, we would refer to this as an asymmetrically split bundle.

The final part of our definition applies to wedge defects that have a distinctly other appearance than split bundles. Example 6 shows the presence of such a wedge defect of the inferior NFL of a 63-year-old glaucoma patient. The wedge defect could be seen with red-free light, there was local cupping of the optic disk and the patient had a superior arcuate scotoma on the visual field test. A wedge defect often appears both on the reflectance image, and on the retardation image. The difference between a split bundle and a wedge defect is that in the latter, the blue area corresponding to the wedge defect is usually sharply demarcated from the adjacent red area. In a split bundle, this transition is more gradual, as seen in examples 2 to 5.

Classifications were performed by two independent observers judging the printouts. An eye had either a split superior or a split inferior bundle, or both, or no split bundle.

Statistical methods We evaluated the inter-observer agreement of the classification using kappa statistics. In interpreting the kappa values we followed the guidelines provided by Altman.[9] To investigate the effect of a split superior bundle on two parameters (smax and symmetry), eyes with a split superior bundle were compared with eyes with no split bundle (table 4-2). A Student's t-test was used to test for differences between the two groups. A subgroup of 200 subjects, that had both eyes imaged with the GDx, was separately analyzed to determine an odds-ratio, and to determine occurrence of split superior bundles per subject (instead of per eye).

Results

The prevalence of split bundles, observed by two different investigators, has been presented in table 4-1. The kappa value for inter-observer agreement was 0.83 (SD = 0.05), which is generally considered to be very good. In case of disagreement, a consensus was reached. Out of a total of 454 eyes, 419 eyes (92.3%) were classified as having no split bundle. In 29 eyes (6.4%), a split superior bundle was observed. In 5 eyes (1.1%), a split inferior bundle was seen and 1 eye (0.2%) had both a split superior and a split inferior bundle.

The mean superior maximum (smax) parameter in subjects with a split superior bundle was statistically significantly lower than the mean smax for the subjects with a single superior bundle (67.2 microns vs 89.9 microns; $p<0.001$; table 4-2). Also, subjects with a split superior bundle had a mean symmetry parameter that was statistically significantly lower as compared to the group with a single superior bundle (0.88 vs 0.98; $p<0.001$).

Of all split bundles, a split superior bundle was most frequent (6.4% of all eyes). To determine in what percentage of *subjects* a split superior bundle occurred, only those 200 subjects included in the study with both eyes were considered (table 4-3). Of these, 24 subjects (12.0%) had a split superior bundle (4 in the left eye, 18 in the right eye and 2 in both eyes). A split superior bundle occurred more often in the right eye than in the left eye which was a statistically significant difference ($p=0.0043$). The odds-ratio of having a split superior bundle in the left eye, relatively to having one in the right eye, was 4.5.

Discussion

We have shown that the prevalence of so-called split nerve fiber layer bundles as measured with the GDx is approximately 8% in a group of 454 healthy eyes. They occurred in about 12% of normal subjects. A split superior bundle was the more common variation. Given the high prevalence in healthy eyes, we believe that a split bundle is a normal variation of the NFL pattern when imaged with the GDx.

In our clinic, we use a so-called integrated approach to interpreting GDx data: we combine quantitative aspects (the various parameters) and non-quantitative aspects (reflectance image, retardation image, double hump pattern) of the data into an integrated evaluation of the GDx results. Being part of the quantitative data, the superior maximum and symmetry parameter are affected. In addition, also non-quantitative aspects of the data are affected. We have illustrated how a split bundle can alter the usual shape of the double hump pattern. Also, we have shown that it should not be confused with a pathologic finding such as a wedge defect, which, at the moment, can only poorly be detected quantitatively with the current software. Thus, recognizing a split bundle as a physiological finding is important when interpreting GDx results, and may increase the specificity of the clinician working with the GDx.

We realize that whether there is an anatomical basis for what we call a (split) 'bundle', would still need histologic confirmation in our subjects. However, until that question can be adequately addressed, clinicians that use the GDx will continue to be confronted with split bundles, and will need to make sense of them. Our paper does not try to explore the human retinal anatomy, but is only meant to help clinicians towards a better interpretation of GDx results. Interestingly, an expert on red-free fundus photography (Dr. HA Quigley, personal communication) was not surprised by our findings. He told us that arcuate bundles appear split on red-free fundus photography in some cases.

To investigate whether a split bundle is not an early sign of glaucoma, all subjects with split bundles have been included in a long-term follow-up study.

Of all the split bundles, a split superior bundle was the more common variation. We have no explanation for this. Also, we found split bundles to occur more frequently in the right eye than in the left eye. This could be due to measurement artifact or coincidence. Although most split superior bundles occurred unilaterally, having a split bundle in one eye increases the odds of having a split bundle in the fellow eye.

The double hump pattern in patients with a split superior bundle looks similar to the optical coherence tomography graph of normal control subjects as has been presented by Pieroth and coworkers.[10] It is unclear whether this shouldering of the OCT graph is related to the split bundles we have described.

As the GDx has been developed over the last years, parameters have been introduced to quantitatively summarize the data. Since a split bundle does not appear to indicate disease, it would be useful to develop an algorithm that can correct the affected parameters, and that can discriminate between split bundles and wedge defects. Until then, it seems that parameters such as symmetry and superior maximum are less reliable in subjects with a split superior bundle. The clinician is advised to rely heavier on the non-quantitative part of the GDx data in these cases.

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Table 4-1. Prevalence of split Nerve Fiber bundles in 454 eyes of 254 subjects.

	no split	superior split	inferior split	superior & inferior split	total
number of eyes (observer #1)	421	29	2	2	454
number of eyes (observer #2)	413	37	4	0	454
consensus	419	29	5	1	454
%	92.3%	6.4%	1.1%	0.2%	100%

Table 4-2. Smax and Symmetry parameters in subjects with a split superior bundle.

	Single superior bundle	Split superior bundle	difference	95 % CI	p-value
Smax (m)	89.9	67.2	22.7	20.2 ; 25.1	<0.001
Symmetry	0.98	0.88	0.10	0.055 ; 0.15	<0.001

The mean superior maximum (smax) and the mean symmetry parameters in subjects with a split superior bundle, as compared to subjects with a single superior bundle. The unit of the smax is μ. Also, the difference between these two values, its 95% Confidence Interval (95% CI), as well as the p-value corresponding to the statistical difference of this difference have been given.

Table 4-3. Prevalence of split superior bundles in subjects who had both eyes imaged (n=200)

		SPLIT SUPERIOR OD		
		yes	no	total
split superior OS	yes	2	4	6
	no	18	176	194
	total	20	180	200

In this table only superior split bundles are considered in those 200 subjects where images could be obtained in both eyes. Subjects had either a split superior bundle (in the right eye or in the left eye, or in both eyes) or no split superior bundles.

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